Regional variation in energy storage strategies in American glass eels from Eastern Canada

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Abstract

Energy status was analyzed in glass eels captured during two early waves of arrival at the mouths of the Mersey River, Nova Scotia, Canada (MR), and Grande-Rivière-Blanche, Québec, Canada (GRB), and according to their salinity preference (freshwater, brackish, or saltwater). Glass eels captured in the GRB estuary were larger, more pigmented, and exhibited higher whole-body glycogen, phospholipid, and sterol and wax ester contents. Those from MR had a higher condition index and a higher whole-body triacylglycerol content, suggesting different patterns of storage and/or use of energy reserves. Within a river, a delay of two weeks in estuarine arrival was characterized by significantly lower energy reserves. No differences in energy storage were observed according to salinity preference. Thus, the results revealed the occurrence of different energy storage strategies according to glass eel migration distance and duration, but not according to salinity preference.

Key words: American glass eels, ecotypes, energy storage strategy, upstream migration, body lipid content

1. Introduction

Numerous biological studies have postulated that bioenergetic constraints have shaped migratory strategies for a wide variety of taxa including fishes (Bernatchez and Dodson, 1987; Schultz and Conover, 1997; Jonsson and Jonsson, 1998; Slotte, 1999; Stockwell

and Johnson, 1999; Morinville and Rasmussen, 2003; Bureau Du Colombier et al., 2007; Busch et al., 2011; Hasler et al., 2012), birds (Johnston and McFarlane, 1967; Wiens and Innis, 1974), and insects (Roff, 1991; Rankin and Burchsted, 1992). In euryhaline fishes, migration is one of the most energetically demanding physiological processes (Gross et al., 1988).

American eel *(Anguilla rostrata,* Lesueur 1817*)* must perform extensive migrations during their life cycle. The leptocephalus larvae are carried by Gulf Stream currents for more than 3800 km from the spawning area in Sargasso Sea to the northern portion of their distribution range in coastal regions of Canada (McCleave, 2001; Tesch, 2003). At an overall mean age of 7–9 months, American eels metamorphose into glass eels, which are considered to be the recruitment stage. This major biological transformation triggers the estuarine migration (e.g., Tesch, 2003). Once they reach estuarine areas, glass eels may migrate upstream in rivers (migratory) or settle in salt or brackish water (residents) for feeding (Lamson et al., 2006; Jessop et al., 2008).

While American eel migration has been the subject of numerous studies, clear evidence for facultative catadromy (non-obligatory trophic migration to fresh water) has only recently been documented. Tsukamoto et al. (1998) were the first to describe a “sea eel” ecophenotype. Daverat et al. (2006) later reported six different patterns of habitat use in temperate eel species, i.e., *Anguilla rostrata, A. anguilla, and A. japonica*. In eastern Canada, many studies have also demonstrated the presence of different migratory patterns in *A. rostrata* (Cairns et al., 2004; Lamson et al., 2006; Thibeault et al., 2007; Jessop et al., 2012; Clément et al., 2014).

The occurrence of facultative catadromy means that eels may exhibit intra-specific variation in physiological capacities to cope with the different environmental conditions that are encountered. In European eel, facultative catadromy has been partly explained by variation in the threshold reaction norm to individual energetic status (Edeline et al., 2006; Edeline 2007; Bureau Du Colombier et al., 2011). Thus, individuals most likely to settle in a saltwater habitat (hereafter saltwater ecotype) are characterized by a low condition factor and low thyroid activity but a high level of growth hormone secretion. Such an endocrine profile results in low locomotor activity, decreased sensitivity to odours, low negative rheotaxis, a preference for saltwater, faster growth rate, and settlement in saltwater (Edeline et al., 2005a, 2005b, 2006; Edeline, 2007). In contrast, individuals most likely to settle in fresh water (hereafter freshwater ecotype) are characterized by a high energetic status and high thyroid activity but a low level of growth hormone secretion, which leads to high locomotor activity, high sensitivity to odours, high negative rheotaxis, a preference for fresh water, and a lower growth rate.

Energy availability can be a limiting factor in migration, particularly in species that do not feed during migration or subsist on energetic reserves, like lipids, accumulated by the preceding stage (e.g., *Alosa sapidissima*: Leonard and McCormick, 1999). Glass eels may not feed until their entry into estuaries (Charlon and Blanc, 1983; Desaunay and Guerault, 1997). Thus, to sustain their energetic demand, glass eels will catabolize the energy stored by the leptocephali during their ocean migration (*A. japonica*: Kawakami et al., 1999; *A. rostrata*: Tesch, 2003). Leptocephali feed on particulate organic matter such as marine snow, zooplankton fecal pellets, gelatinous zooplankton, larvaceans, and

92 discarded appendicularian houses (Pfeiler, 1999; Riemann et al., 2010; Miller et al.,
93 2013). The nutritional condition of leptochephali, which is affected by food availability,
94 global warming trends, and local continental factors, will affect glass eel survival and
95 development (*A. rostrata, A. anguilla*: Desaunay and Guerault, 1997; *A. japonica*:
96 Kawakami et al., 1999; *A. rostrata, A. anguilla, A. japonica*: Munk et al., 2010; Knights,
97 2003).
98 In Canada, American eel is a threatened species (COSEWIC, 2012). Furthermore, the
99 recruitment decline in the St. Lawrence system is far more drastic than on the Atlantic
100 coast, with a reduction of more than 99% from 1986 to 2012 in the St. Lawrence system
101 compared to 39% from 1993 to 2009 in Scotia-Fundy (Cairns et al., 2014). This is of
102 major concern because this portion of the species, which is panmictic (Côté et al., 2013),
103 is believed to have been the major source of female reproductive output before this
104 decline (Castonguay et al., 1994; Cairns et al., 2007; Dutil et al., 2009). Edeline (2007)
105 developed a theoretical model based on the "conditional evolutionarily stable strategy"
106 model, which predicts that the proportion of migrants in the population would decrease
107 with decreased overall recruitment.
108 As stated above, different migratory patterns have been observed in Atlantic Canada. In
109 the Maritimes, the presence of a saltwater ecotype has been described (Cairns et al.,
110 2004; Jessop et al., 2012; Clément et al., 2014), while the presence of different ecotypes
111 has not yet been investigated in the St. Lawrence estuary. One hypothesis would be that
112 sample origin defines the presence of freshwater vs. saltwater ecotypes. Alternatively,
113 based on Edeline (2005), it could be that ecotypes are represented in both samples but are
114 only revealed by salinity preference experiments. Boivin et al. (2015) compared salinity

preference among glass eels captured in four different rivers (two in Nova Scotia and two in Québec) and showed that, among those that showed salinity preference, 60 to 75% of glass eels displayed similar preference for fresh water regardless of their geographic origin. However, controlled experiments have revealed the occurrence of growth variations and gene expression as a function of salinity conditions among regions, supporting the hypothesis of spatial variation in selection between glass and yellow eels from different origins even though the species is panmictic (Côté et al., 2009, 2014, 2015; Boivin et al., 2015). Moreover, a recent population genomics study by Pavey et al. (2015) recently provided strong evidence for genetic differentiation between yellow eels occupying brackish vs. eels occupying freshwater.

In this context, the objectives of this study were to determine how the energetic profile would influence migration distance (Nova Scotia *vs.* St. Lawrence estuary). We also tested the hypothesis that differences in condition and energy status would determine salinity preference, with high energy reserves being associated with a preference for fresh water. To do so, we examined glycogen and lipid profiles, two biochemical sources of energy used by different stages of fish larvae (*Sciaenops ocellata*: Vetter et al., 1983; *A. sapidissima*: Leonard and McCormick, 1999; *Onchorhynchus kisutch* and *O. tshawytscha*: Trudel et al., 2005; *Pseudopleuronectes americanus*: Fraboulet et al., 2010; 2011). Lipid class characterization is a powerful tool to identify energy reserves when energetic macromolecules are not clearly identified. It has been widely demonstrated that triacylglycerol (TAG), which is made up of three fatty acids that esterify to a glycerol backbone, is a common storage lipid in fishes, but other neutral lipids like wax ester, which have only one fatty acid that esterifies to a fatty alcohol, could play a role (Budge

et al., 2006). Such information will improve our understanding of diadromous behaviour and the migration strategy used by American glass eels. This will allow appropriate management strategies to be developed that—it is hoped—will lead to stock recovery.

2. Material and methods

2.1 Fish collection

Glass eels were captured (n = 4822) in the estuaries of two east coast Canadian rivers: from a commercial elver fishery in the Mersey River, Nova Scotia, on 26-28 March and 20-21 April 2012 and from Grande-Rivière-Blanche, Québec, on 2-6 and 18-21 June 2012 (Figures 1 and 2). These periods represent the early arrival of glass eels in this area (Côté et al., 2013). Glass eel captures began two hours before the nighttime high tide and lasted for three hours. Samplers waded into river mouths and captured eels using dip-nets and headlamps. Glass eels were transferred by car to the wet-lab facility at Maurice-Lamontagne Institute (IML; Fisheries and Oceans Canada) in buckets containing water from the estuary. The introduction and transfer of glass eels between provinces were done under conditions specified in the license obtained from Fisheries and Oceans Canada. Salinity preference tests were done upon arrival at IML and individuals tested by Boivin et al. (2015) were used in the present study.

Following salinity preference determination (see Boivin et al., 2015 for a complete description of the methodology), a total of 120 glass eels were sampled for analyses: 30 glass eels from each sampling site and sample date (total of 60 for each river) including 10 with a preference for fresh water, 10 with a preference for brackish water, and 10 with

a preference for salt water for each river and each sample date (Figure 2). Fish were individually anaesthetized in an aqueous solution of MS-222 (0.68 mM l^{-1} of ethyl 3-aminobenzoate methanesulfonate; Sigma-Aldrich) in a Petri dish. Total body length (from the tip of the snout to the tip of the caudal fin; ± 1 mm) and wet mass (± 1 mg) were measured. Pigmentation stage was identified according to Haro and Krueger (1988). Glass eels were rinsed with brackish water, gently blotted dry, and transferred to 1.5 ml Eppendorf tubes that were immediately placed on dry ice. Samples were kept frozen (-80°C) until analysis.

2.2 Homogenates

For each sample, the whole glass eel was cryogenically ground using a stainless 12 mm ∅ grinding bead in a Mixer Mill MM 400 (Retsh, Germany). The grinding bead was immerged for 30 s in liquid nitrogen before being transferred to the Mixer Mill for 1 min at a frequency of 12 Hz; each individual was ground twice. The homogenization equipment was cleaned with ethanol and rinsed with MilliQ water between samples. Ground tissue was transferred to Eppendorf tubes containing 0.8 ml ice-cold 10 mM phosphate buffer (pH 7.4) and stored at -80°C.

2.3 Analyses

The Le Cren condition index (Kn), which is independent of size (Le Cren, 1951), was used because American glass eel growth is not isometric ($a_c \neq 3$) (Figure 3). The index is calculated as follows:

$$Kn = W_m (aL^b)^{-1}$$

where W_m is wet mass, L is total length, and a and b are empirically determined constants. The a and b constants were obtained by fitting a linear regression through log10 transformed length and mass data, which resulted in the following equation:

$$\log_{10} Wm = -4.95 + 2.33 \log_{10} L;\ r^2 = 0.47;\ n = 195.$$

Glycogen was measured using the quantitative enzyme assay described by Carr and Neff (1984) using a microplate reader (VMAX, Molecular Devices) at 414 nm. Lipids were extracted according to the Folch et al. (1957) procedure modified by Parrish (1999). The relative proportions of the different lipid classes (hydrocarbons [HC], sterol [SE] and wax esters [WE], ketones [KET], triacyglycerols [TAG], free fatty acids [FFA], acetone-mobile polar lipids [AMPL], and phospholipids [PL]) were determined using an Iatroscan Mark-VI analyzer (Iatron Laboratories Inc., Tokyo, Japan) and were developed in a four-solvent system (Parrish, 1987, 1999). Lipids were extracted from 0.6 ml of homogenate with 4 ml of a chloroform–methanol (2:1) solution in a glass Dounce tissue homogenizer followed by the addition of 1.5 ml of KCl. The organic phase was collected after each of two centrifugations (2 min at 2000 rpm), evaporated under nitrogen flux at 35°C, resuspended in 0.250 ml of chloroform, and stored at -80°C. Extracts and the standard were spotted onto silica gel-coated chromarods (SIII; Shell USA), and lipid classes were separated using four different solvents and then quantified by thin-layer chromatography using flame ionization detection (Iatroscan MK-6, Shell USA). Lipid class peaks were quantified with PeakSimple software version 3.21 (SRI, Inc.), and lipid classes were identified and quantified using standard calibration curves obtained for each standard (Sigma Chemicals, Inc.). In addition, each analysis run included one composite standard in one of the 10 rods available, as suggested by Parrish (1987). Lipid classes were

measured as µg/mg of wet mass, summed to obtain total lipids, and expressed as percentage of total lipids.

2.4 Statistical analyses

The effect of river and date of capture on wet mass, length, Kn, glycogen concentration, total lipids concentration were analyzed with two-way ANOVAs ($\alpha = 0.05$) using STATISTICA v6.0 software (www.statsoft.com). Significant differences were identified with Tukey's multiple comparison tests ($p < 0.05$). Normality and homoscedasticity of data were verified with the Kolmogorov–Smirnov and Levene tests, respectively. The effect of salinity (experimental data) was analyzed using one-way ANOVA for glass eels originating from the same river and same date of capture to isolate the effect of salinity. Three-way ANOVAS could not be used because of capture differences from site to site. Quantitative pigmentation index data were analyzed with the nonparametric Kruskal–Wallis test. Lipid classes were analyzed separately using three-way PERMANOVA ($p < 0.05$) with 9999 permutations based on a Bray-Curtis matrix (river, date of capture, salinity preference). A posteriori comparisons were done using a PERMANOVA pairwise test. To analyze the similarity between profiles, non-metric multi-dimensional scaling (n-MDS) and Simper analyses were performed with Primer 6.1.1.12 and PERMANOVA+ 1.0.2. Percentage data (lipid classes) were arcsine transformed (Sokal and Rohlf, 1995). When significant effects were found, variations of these effects were illustrated by two-way ANOVAs on arcsine-transformed data. Relationships between Kn and four proxies of energy content (glycogen, total lipids, triacyglycerols, and sterol and

wax esters; expressed in μg per mg of wet mass) were analyzed by linear regression (α = 0.05).

3. Results

3.1 Comparison between rivers and dates of capture

Date of capture or origin did not influence the wet mass or total lipid content of individuals (Table 1). However, those that arrived later were more pigmented (Figure 4A; $p < 0.001$), and glass eels from GRB were longer (Table 1) and more pigmented (Figure 4B; $p = 0.027$). Moreover, the Kn of glass eels entering MR was higher than that of eels entering GRB. Kn increased with time of capture in MR but not in GRB (Table 1).

The glycogen content of MR glass eels was similar between capture dates. However GRB glass eels captured during the first sampling period had significantly more glycogen than those captured two weeks later (Table 1), and their glycogen content was significantly higher than MR for both dates.

PL and TAG were the two main lipid classes present in *A. rostrata* glass eels followed by ST and SE-WE (Table 1). TAG, PL, and SE-WE altogether explained more than 75% of the dissimilarities between river and date of capture (Table 2); TAG alone explained near 40%. Indeed, TAG were significantly higher in glass eels from MR than in those from GRB, with correspondingly lower PL and SE-WE contents since the content of total lipids was similar between origins (Table 1). For both rivers, glass eels that entered the estuary earlier in the season had significantly more TAG and SE-WE than those arriving later (Table 2).

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253 *3.2 Comparison among glass eels exhibiting different salinity preferences*

254 Few differences were observed among glass eels exhibiting different salinity preferences.
255 For each river and for each date of capture, wet mass and total lipid content were similar
256 for glass eels with different salinity preferences (Table 3). Significant differences in
257 length for glass eels with different salinity preferences were only observed in glass eels
258 from MR during the second sampling session, with glass eels exhibiting a preference for
259 freshwater being longer than those with a preference for brackish water. In glass eels
260 from MR arriving earlier, those that preferred salt water had a higher Kn than those
261 preferring brackish water. Moreover, those preferring fresh water had higher glycogen
262 content than those preferring brackish water (Table 3). No differences in lipid class
263 profiles were observed (Table 2).

264

265 *3.3 Condition index and energy reserves*

266 Overall, Kn was significantly correlated with different proxies of energy content, but
267 correlation coefficients were low (Figure **5**). Surprisingly, Kn was negatively correlated
268 with glycogen and SE-WE contents (Figure **5**A; **5**D). There was no relationship between
269 total lipid content and Kn (Figure **5**B). However, Kn was positively correlated with TAG
270 content (Figure **5**C).

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4. Discussion

The aim of this study was 1) to verify whether differences in energetic status might be related to differences in migration distance, migration duration, or salinity preference of glass eels and 2) to determine whether the energetic status can reveal information on the physiological processes underlying the differentiation of marine or freshwater ecotypes. The results revealed the occurrence of different energy storage strategies according to migration distance and duration, but not according to salinity preference.

4.1 Comparisons between rivers and dates of capture

Within a river, wet mass was similar between capture dates, suggesting that glass eels arriving later in the river estuary did not experience greater migration costs; this was true for both MR and GRB glass eels. Dutil et al. (2009) estimated that one to two months were required for glass eels to transit from Cabot Strait to the St. Lawrence estuary, and this is exactly the delay observed between captures in MR and GRB. However, glass eels at GRB were longer and more pigmented than those from MR, indicating that they were nearer the elver stage and perhaps beginning the transformation to the yellow eel stage. It has been shown that American glass eel length increases with migration distance (Haro and Krueger, 1988; Laflamme et al., 2012) and that upstream migration is more costly for smaller individual (Weihs, 1977). The results from the present study agree with those obtained in the more southern part of this species' distribution area, where a very low pigmentation index was found in glass eels entering river estuaries in Florida (Sullivan et al., 2009). This is not specific to American eel: numerous studies have shown that glass

298 eels of different species are older and longer at recruitment relative to distance from the
299 breeding site (European glass eels: Naismith and Knights, 1988; Japanese glass eels:
300 Tsukamoto and Umezawa, 1990; American and European glass eels: Wang and Tzeng,
301 2000).
302 The results indicate that wet masses were similar between capture dates. Similarly,
303 Bureau du Colombier et al. (2011) observed no differences in wet mass in recently
304 captured and starved European glass eels following 28 days spent at different salinities.
305 Moreover, there is a general pattern in migratory fishes that those species (and
306 populations within species) that make difficult and long migrations are larger and use
307 their energy reserves more efficiently than those that make short migrations (Bernatchez
308 and Dodson, 1987). This could possibly explain the observed differences between GRB
309 and MR glass eels. The other explanation would be that feeding resumed with the
310 development of pigmentation (Tesch, 2003).
311 In European silver eels, migration success depends on the amount of lipids stored during
312 the growth phase (Boëtius and Boëtius, 1985). However, while we found no significant
313 difference in total lipids, there were significant differences in lipid class composition
314 depending on the river of origin, notably in the relative proportions of TAG followed by
315 PL. Neutral lipids, and especially TAG, are generally the preferred source of metabolic
316 energy in marine fishes for growth, reproduction, and swimming, particularly for the first
317 ontogenic stages (Tocher et al., 2008). Lipids can be obtained from either external or
318 internal body sources, and they may or may not be correlated to body mass. In the present
319 study, neither total lipids nor mass varied with the river of origin, so glass eels were
320 either eating during their migration to river estuaries or they had sufficient reserves to

sustain their migration to the sampling sites. However, since our results are expressed relative to wet mass, they cannot take into account the possible effects of changes in water content. Another hypothesis is that glass eels captured in GRB could have had more lipids at the beginning of migration that would have been used during their transit from the Sargasso Sea to the St. Lawrence estuary. Indeed, GRB glass eels arrived one month later than the MR eels.

If it is assumed that glass eels do not eat during the estuarine migration (Charlon and Blanc, 1983; Tesch, 2003), lower energy reserves in GRB glass eels would be expected. In fact, there was a lower proportion of TAG, the main lipid reserve. This is consistent with previous studies done on unfed larvae that showed either lipid depletion or specific TAG depletion with time (e.g., Glencross, 2009). TAG constitutes a pool of energy reserves in marine fishes and is considered as the most efficient nutrient for maximizing energy production (e.g., Glencross, 2009). Bernatchez and Dodson (1987) showed that energy efficiency increases with increased migratory distance, thus the preferential use of TAG at GRB could be explained by a greater efficiency of energy use in those glass eels migrating further north.

The relative percentages of the different lipid fractions vary greatly in starved larvae depending on species, life stage, and environmental conditions (e.g., fishes: Rainuzzo et al., 1997; Turchini et al., 2009). In unfed *Solea senegalensis* larvae, weight loss is due to lipid catabolism and lipid depletion since these larvae preferentially consume neutral lipids during development (Mourente and Vázquez, 1996). Unfed larvae of Atlantic bonito, *Sarda sarda,* gained dry mass and lost lipid content, mainly TAG and SE, during development (Ortega and Mourente, 2010). In turbot, *Scophthalmus maximus*, a rapid

344 decrease in lipids with simultaneous reduction in the dry weight occurred in unfed larvae,
345 and SE and TAG fractions were preferentially catabolized (Rainuzzo et al., 1997). Lipid
346 depletion with specific catabolism of TAG was also observed during the migration of
347 starved lamprey larvae, *Petrmyzon marinus* (Kao et al., 1997).
348 Since TAG were preferentially used, it is somewhat surprising that there was no change
349 in wet mass. In Japanese glass eels, wet weight was shown to be correlated to the lipid
350 content of the peritoneal cavity, and this relationship was suggested as a useful way to
351 estimate nutritional status (Kawakami et al., 1999). The same authors also observed that
352 glass eels that arrived first at river mouths had higher mass than those that arrived two
353 months later. A correlation between the percentage of body fat and eel size was also
354 found in adult American eels (Gallagher et al., 1984), and lipid percentage was higher in
355 larger European eels than in smaller ones (Degani, 1986). In the present study, the
356 replacement of storage lipids by structural ones may explain the absence of wet mass
357 differences.
358 The PL and SE-WE fractions were higher in GRB glass eels. In early juvenile fish, PL
359 improve growth as well as survival rate and stress resistance (Glencross, 2009; Tocher et
360 al., 2008). PL are mainly used as structural elements of biological membranes, so this
361 could explain why this fraction is more important in more developed and longer glass
362 eels. In copepods, reef corals, and several fishes, WE can be used as metabolic energy
363 reserves (Lee et al., 1971; Rahn et al., 1973; Figueiredo et al., 2012), and WE metabolism
364 may be linked with TAG metabolism since tryacylglycerol lipases act on WE (e.g.,
365 Tocher, 2003). SE fractions have not been extensively studied in fishes, but they could be
366 catabolized as energy reserves in the same way as TAG or WE (e.g., Ortega and

Mourente, 2010) while also being structural components of the cell architecture. Similar trends for SE-WE and PL fractions were observed, i.e., a greater proportion in more developed GRB glass eels along with a decrease in TAG, thus it is suggested that the changes in proportions observed in the present study would probably be more related to the structural role of SE.

Glycogen content was more than twice as high in GRB glass eels, suggesting that they preferentially oriented their metabolism to glucose conservation. In European glass eels, Degani et al. (1986) showed that lipids are preferred to carbohydrates to sustain metabolic needs. In adults, Larsson and Lewander (1973) revealed the utilization of liver and muscle triglycerides as energy sources and for the stimulation of gluconeogenesis, both of which increased in later phases of starvation. Moon (1983) suggested a minor role of carbohydrates in the fasting period of immature American eels, as shown by a decline in glycogen phosphorylase activity. Jedryczkowski (1979) and Degani et al. (1986) also showed that glycolysis efficiency in European eel was lower in freshwater during early development based on changes in aldolase activity. Differences in the relative proportion of palmitic acid in fatty acids were identified between freshwater and marine fishes (Ackman, 1967), thus glass eels from GRB may have a strategy close to freshwater fishes. However, fatty acid analyses are needed to confirm this.

Glucose is essential to sustain oxidative metabolism in specific cells such as nervous tissue. TAG metabolism may help maintain glucose levels through gluconeogenesis and glycogen synthesis pathways, or glucose stocks may be preserved through energy production sustained by fatty acids or ketones to the ß oxidation pathway (e.g., Tocher, 2003; McCue, 2010). Thus, having high glycogen storage coupled with a reduced TAG

390 proportion seems plausible. As reviewed by McCue (2010), the ability to recover
391 glycogen storages could differ as starvation progresses or be linked to a difference in the
392 ability to endure a greater period of starvation and to prioritize metabolic costs in specific
393 organs and tissues.
394 The presence of differences in energy stores deserves further investigation. Indeed,
395 despite panmixia, a latitudinal cline in allele frequencies was observed in genes encoding
396 for enzymes related to energetic metabolism, including sorbital dehydrogenase, alcohol
397 dehydrogenase, and phosphosehexose isomerase, in American glass eels captured from
398 Florida to Newfoundland (Koehn and Williams, 1978). More recently, Gagnaire et al.
399 (2012) identified several genes that had spatially varying selection associated with habitat
400 heterogeneity (three genes associated with lipid metabolism, two with saccharide
401 metabolism, three with protein biosynthesis, three with defense response, and one with
402 molecular function). This observation suggests that glass eels colonizing different areas
403 of the geographical range, which are characterized by different physico-chemical
404 characteristics, are exposed to differential patterns of selection. Moreover, adaptation to
405 the water temperature gradient encountered in river estuaries from south to north would
406 be relevant in variants of genes implicated in metabolism (Gagnaire et al., 2012). More
407 recently, Pavey et al. (2015) performed a genome-wide association study that
408 demonstrated a polygenic basis that discriminates American eels from freshwater and
409 brackish water habitats. They found that 331 co-varying loci out of 42,424 were
410 associated with the divergent ecotypes. These 331 SNPs are associated with 101 genes
411 that represent vascular and morphological development, calcium ion regulation, growth
412 and transcription factors, and olfactory receptors. Finally Côté et al. (2014) also showed

413 that gene × environment interactions may explain growth differences between MR and
414 GRB yellow eels since differences were found in the expression of genes related to
415 energy metabolism, energy respiration, growth, differentiation, and development.
416 Within a river, a delay of two weeks in estuarine arrival was characterized by
417 significantly lower energy reserves. In GRB, TAG and glycogen contents were lower in
418 fish captured later in the season while SE-WE increased and body condition, total lipid
419 content, and wet mass remained constant. This again supports the hypothesis of the use of
420 TAG and carbohydrates to sustain metabolism and a structural role for the lipids found in
421 the SE-WE fraction in this particular region.
422 MR glass eels arriving later also showed lower proportions of TAG and higher SE-WE
423 contents, but their glycogen content was similar and Kn was higher than those in GRB
424 glass eels. This indicates a difference in the use of metabolic reserves between the two
425 areas. The patterns of Kn are difficult to explain in the absence of changes in total lipids
426 and a decrease of storage lipids. The use of dry mass to express total lipids could have
427 circumvented this.
428
429 *4.2 Comparison between glass eels exhibiting different salinity preferences*
430 One of the main objectives of this study was to verify if energy status could be associated
431 with habitat selection. A worldwide decline in freshwater eel recruitment is occurring,
432 and settlement in saltwater environments is apparently increasing in American and
433 European eels (e.g., Lambert, 2005; McCleave and Edeline, 2009). Behaviour
434 experiments using MR and GRB glass eels allowed the identification of active glass eels,
435 which had a preference either for freshwater or saltwater, and inactive eels, which had a

preference for brackish water (Boivin et al., 2015). Here, it was tested whether different salinity preferences could be correlated with specific energy status. Indeed, fatty acid requirements (e.g., Glencross, 2009), digestibility, transport, uptake, elongation and desaturation processes, and ß-oxidation of fatty acids (e.g., Turchini et al., 2009) should be considered when looking at body lipid composition, but it may also be affected by abiotic factors including water salinity, temperature, and light (e.g., Dantagnan et al., 2013). Thus, salinity affects fish metabolism (Sampekalo et al., 1992), and differences in energy stores in glass eels could explain the occurrence of different metabolic strategies between the ecotypes considered.

Based on the conditional evolutionarily stable strategy suggested for European eel, in which migration in freshwater or saltwater at recruitment depends on the individual's energetic and thyroid status, freshwater glass eels should have a high energetic status and high thyroid activity, which would result in freshwater preference, low growth rate, and high migratory activity in contrast with saltwater glass eels (American yellow eel: Castonguay et al., 1990; European glass eel: Edeline et al., 2004, 2005a, 2005b, 2007; European elver and yellow eel: Imbert et al., 2008). Then lower energy reserves and larger size in glass eels with saltwater preference would have been expected compared to those preferring fresh water. Not only there was no difference based on salinity preference, but the river differences also did not support this hypothesis for American glass eels since the freshwater ecotype would be expected to be more frequent in GRB and the marine ecotype more frequent on the Atlantic coast (i.e., MR). It should be remembered that energetic status differences in European eel were suggested from condition factor data (Edeline et al., 2006; Bureau Du Colombier et al., 2011). It is

plausible that the dichotomy between freshwater and marine ecotype in our system would be better reflected by geographical differences rather than salinity preferences. Because condition factor did not dffer between rivers, it is very difficult to make such comparisons with data on European eel. However, the present results are consistent with those obtained by Boivin et al. (2015), who observed no relationship between salinity preference and body condition in American eel, but observed differences in growth between origins under controlled conditions.

5. Conclusion

These results on American eel did not support the hypothesis of conditional strategy, i.e., that migration in freshwater or saltwater at recruitment depends on the individual's energetic status. Instead, the presence of higher carbohydrate content and differences in lipid storage and/or use of different lipid classes corroborate the occurrence of genetic differences between habitats and related to sites colonized by glass eels. How differences observed between rivers and dates of capture may affect glass eel survival and recruitment is unknown, but it certainly deserves further attention.

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756

757 **Legends**

758

759 **Figure 1. River estuaries where glass eel were sampled for this study.** Grande-
760 Rivière-Blanche (GRB), 48°47' N, 67°41' W; Mersey River (MR), 44°02' N, 64°42' W.

761

762 **Figure 2. Experimental design**

763

764 **Figure 3. Linear regression of the biometric relationship in American glass eel**
765 **between length (mm) and wet mass (g) on an ln–ln axis.** The figure shows the fitted
766 regression line and 95% confidence intervals (dashed lines); the regression equation,
767 coefficient of determination (r^2), correlation coefficient (r), and p-value are also given.

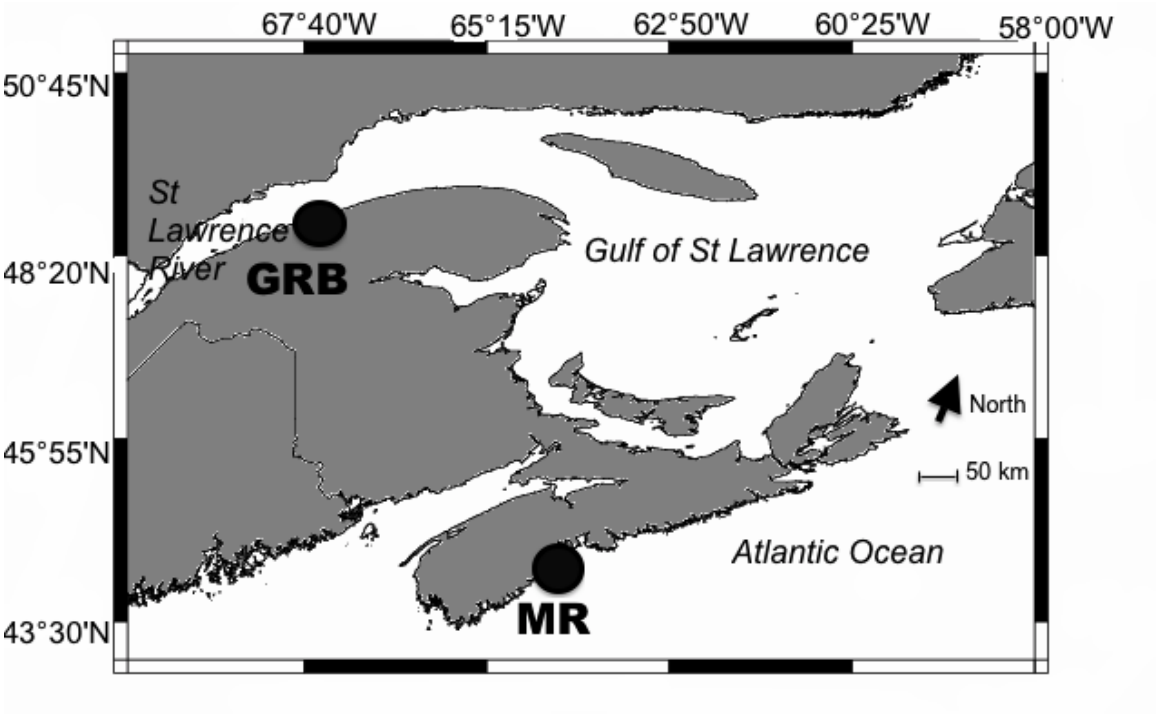
768

769 **Figure 4. Kruskal–Wallis results on pigmentation stage for each date (A) and each**
770 **river (B) represented by boxplot figures.** Asterisks indicate significant differences
771 between rivers or dates of capture. Boxplots show minimum and maximum values, 25–
772 75% rectangles, and the median. GRB: Grande-Rivière-Blanche; MR: Mersey River.

773

774 **Figure 5. Glycogen (A), total lipid (B), tryacylglycerol (TAG) (C), and sterol and wax**
775 **ester (SE-WE) (D) contents in relation to the Le Cren condition index (Kn).** Data are
776 expressed as µg of mg of wet mass. The coefficient of determination (r^2), correlation
777 coefficient (r), and p-values are shown.

778


67°40'W
65°15'W
62°50'W
60°25'W
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50°45'N
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45°55'N
43°30'N
St Lawrence River
GRB
Gulf of St Lawrence
North
50 km
Atlantic Ocean
MR

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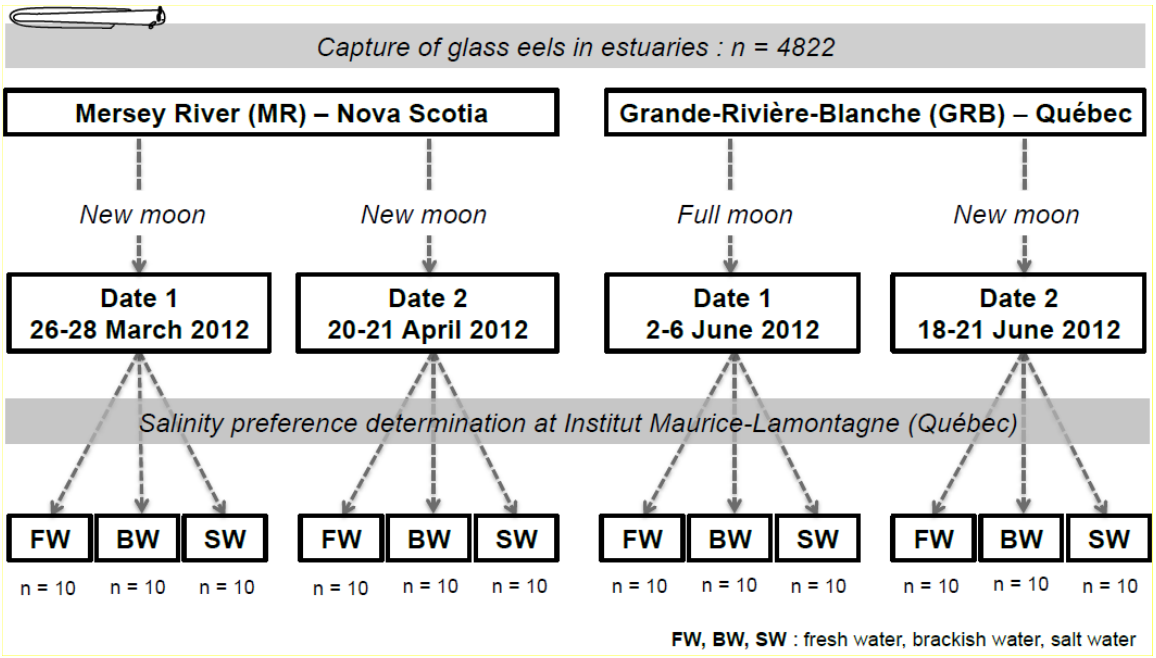
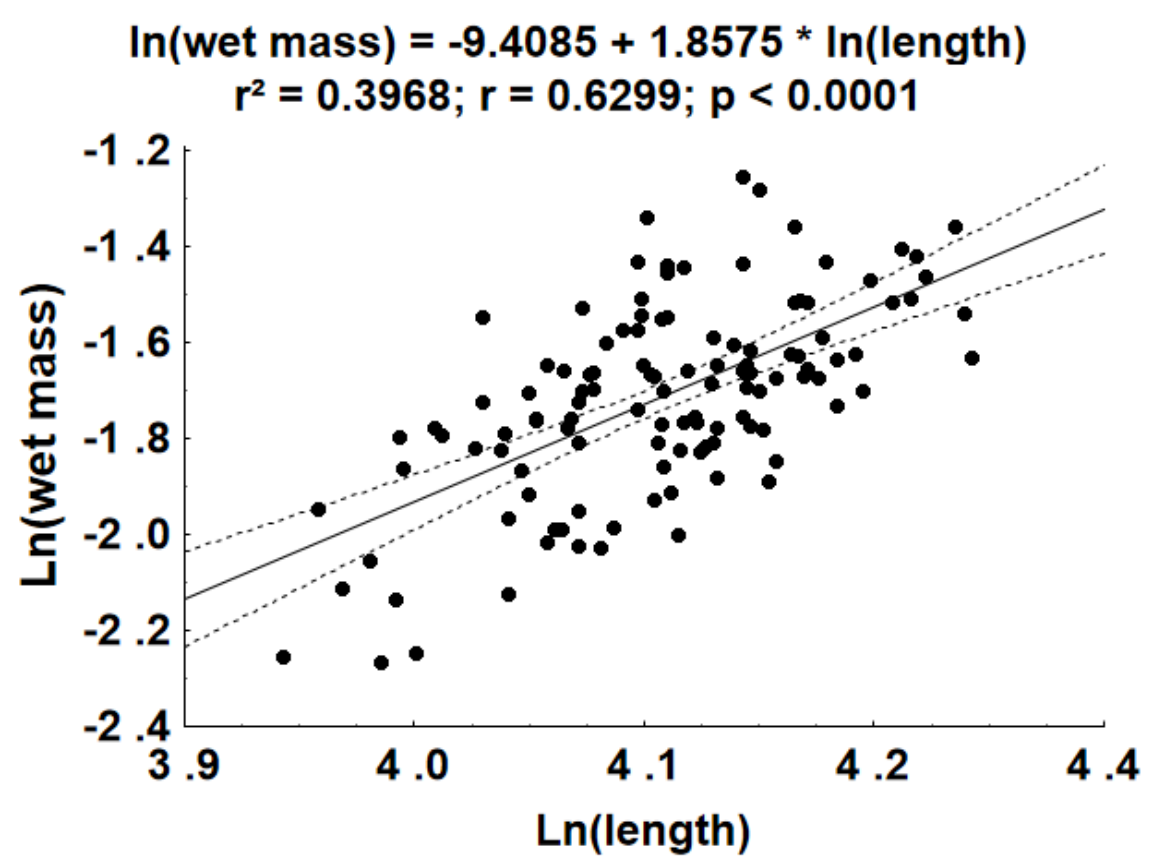
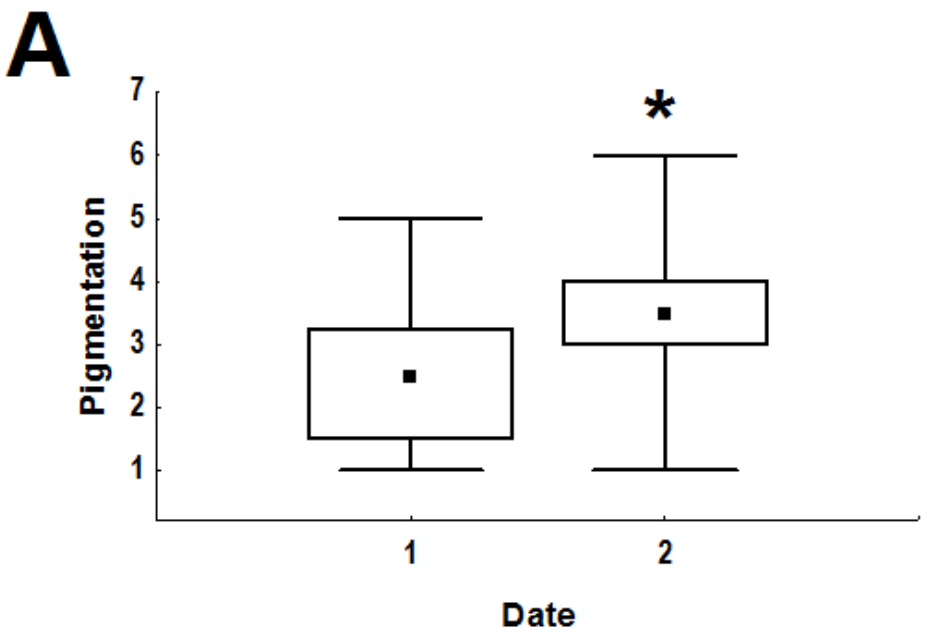
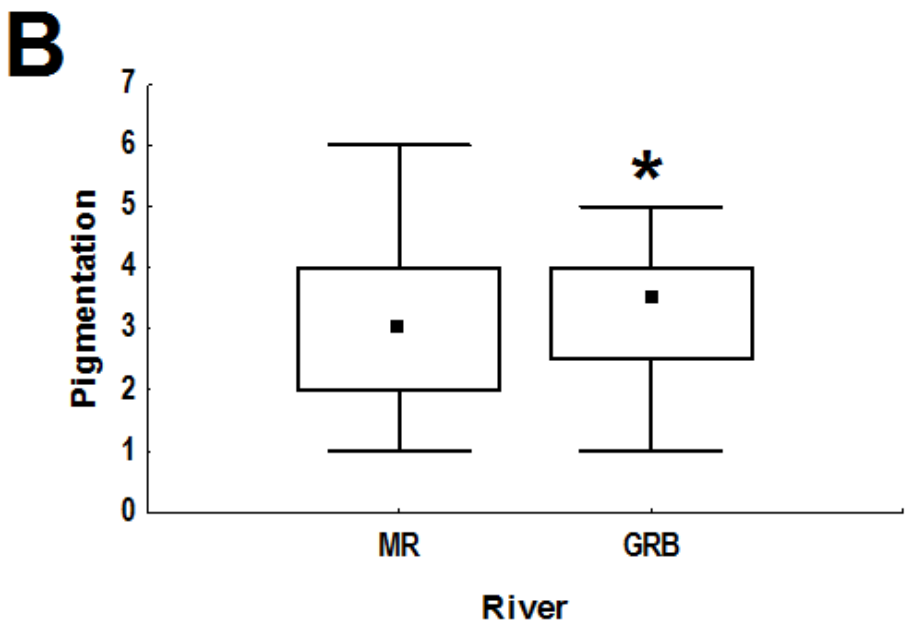

Capture of glass eels in estuaries : n = 4822
Mersey River (MR) – Nova Scotia
Grande-Rivière-Blanche (GRB) – Québec
New moon
New moon
Full moon
New moon
Date 1
26-28 March 2012
Date 2
20-21 April 2012
Date 1
2-6 June 2012
Date 2
18-21 June 2012
Salinity preference determination at Institut Maurice-Lamontagne (Québec)
FW
BW
SW
n = 10
FW, BW, SW : fresh water, brackish water, salt water

781
782

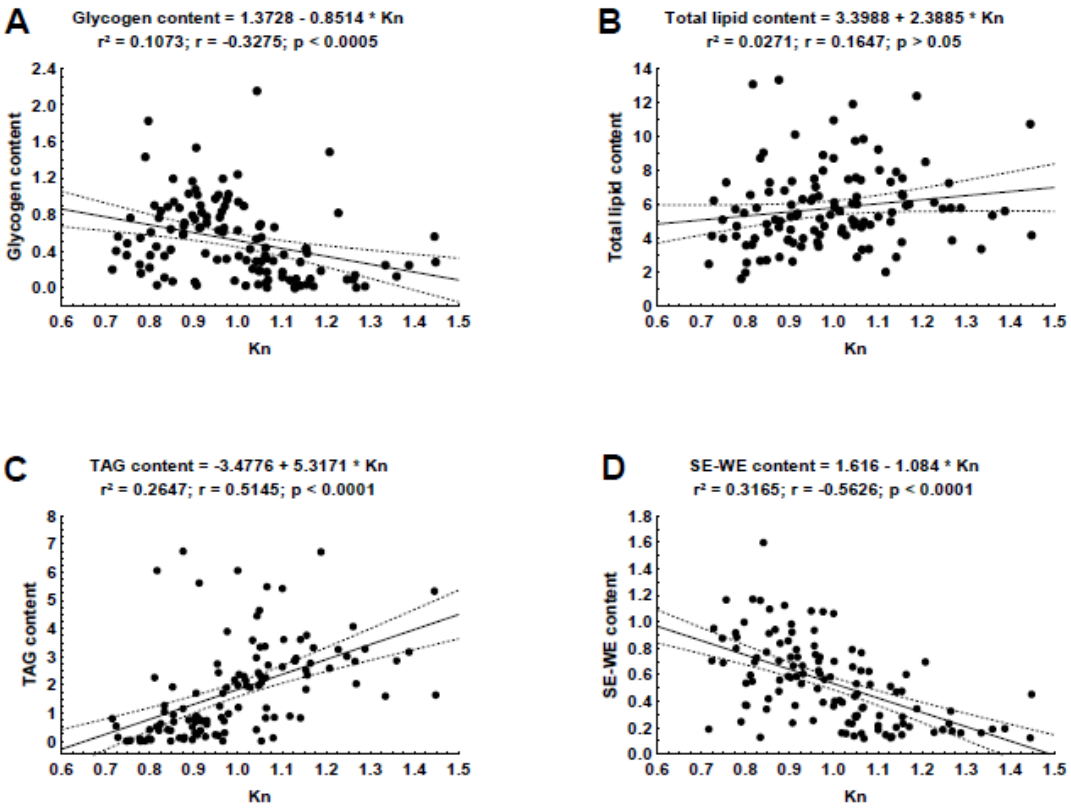

ln(wet mass) = -9.4085 + 1.8575 * ln(length)
r² = 0.3968; r = 0.6299; p < 0.0001
Ln(wet mass)
-1 .2
-1 .4
-1 .6
-1 .8
-2 .0
-2 .2
-2 .4
3 .9
4 .0
4 .1
4 .2
4 .4
Ln(length)

783
784


A
Pigmentation
7
6
5
4
3
2
1
*
1
2
Date


B
Pigmentation
7
6
5
4
3
2
1
0
*
MR
GRB
River

785


A
Glycogen content = 1.3728 - 0.8514 * Kn
r² = 0.1073; r = -0.3275; p < 0.0005
Glycogen content
Kn
B
Total lipid content = 3.3988 + 2.3885 * Kn
r² = 0.0271; r = 0.1647; p > 0.05
Total lipid content
Kn
C
TAG content = -3.4776 + 5.3171 * Kn
r² = 0.2647; r = 0.5145; p < 0.0001
TAG content
Kn
D
SE-WE content = 1.616 - 1.084 * Kn
r² = 0.3165; r = -0.5626; p < 0.0001
SE-WE content
Kn

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787 **Table 1. Results of two-way ANOVA (River, Date, River × Date) on wet mass (g), length (mm), Le Cren condition index (Kn), glycogen**
788 **content (µg mg^{-1} of wet mass), total lipids (µg mg^{-1} of wet mass), and relative proportions (% of total lipids) of triacylglycerols (TAG),**
789 **phospholipids (PL), sterol and wax esters (SE-WE), and sterols (ST) in glass eels captured in two rivers (Mersey River: MR, Grande-**
790 **Rivière-Blanche: GRB) at first arrival (MR 1, GRB 1) and at the next spring tide (MR 2, GRB 2).** Mean ± SE. Bold characters indicate
791 significant differences between rivers, bold italic characters indicate significant differences between dates of capture, and different superscript
792 letters indicate significant differences when significant interactions between factors were present. ns: no significant difference.

	MR 1 N=30	**MR 2** N=29	**GRB 1** N=30	**GRB 2** N=29	**Effect River, Date, or River × Date**
Wet mass	0.18 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	Ns
Length	60.1 ± 0.70	61.28 ± 0.64	**65.49 ± 0.58**	**65.56 ± 0.72**	GRB > MR, $p < 0.0001$
Kn	1.04 ± 0.03^{b}	1.13 ± 0.03^{a}	0.92 ± 0.02^{c}	0.89 ± 0.02^{c}	River × Date, $p < 0.05$
Glycogen	0.23 ± 0.04^{c}	0.28 ± 0.05^{c}	0.88 ± 0.07^{a}	0.70 ± 0.07^{b}	River × Date, $p < 0.05$
Total lipids	7.91 ± 1.93	6.32 ± 0.46	5.85 ± 0.36	4.86 ± 0.33	Ns
TAG (%)	***45.17 ± 1.98***	**42.19 ± 1.83**	***15.03 ± 1.96***	7.88 ± 1.81	MR > GRB, $p < 0.0001$; 1 > 2, $p < 0.01$
PL (%)	28.80 ± 1.11	28.08 ± 1.58	**48.43 ± 1.24**	**50.48 ± 1.42**	GRB > MR, $p < 0.0001$
SE-WE (%)	6.53 ± 1.59	***10.39 ± 0.62***	**13.29 ± 0.63**	***15.75 ± 0.55***	GRB > MR, $p < 0.0001$; 2 > 1, $p < 0.05$

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794 **Table 2. Results of three-way PERMANOVA, average similarity, average dissimilarity, and dissimilarity contributions greater than**

795 **10% in lipid profiles.** River: Mersey (MR), Grande-Rivière-Blanche (GRB); Date of capture: first week of arrival and two weeks later; salinity

796 preference: fresh, salt or brackish water. TAG: tryacylglycerols; PL: phospholipids; SE-WE: sterol and wax esters. Bold: significant differences.

Source	Df	Pseudo-F	P (perm)	Average similarity (%)	Average dissimilarity (%)	Dissimilarity contribution (>10%)
River	**1**	**260.6**	**0.0001**	**MR (82.61) GRB (84.47)**	**40.43**	**TAG (40.30) PL (26.46) SE-WE (12.61)**
Date	**1**	**8.84**	**0.0005**	**Date 1 (73.05) Date 2 (70.14)**	**28.73**	**TAG (39.00) PL (25.66) SE-WE (14.39)**
Salinity	2	2.00	0.0922	-	-	-
River × Date	1	0.64	0.5598	-	-	-
River × Salinity	2	0.38	0.8438	-	-	-
Date × Salinity	2	0.33	0.8748	-	-	-
River × Date × Salinity	2	1.51	0.1916	-	-	-
Residuals	106	75.31				

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798 **Table 3. ANOVA results for salinity preference for each river and date of capture on wet mass (g), length (mm), Le Cren condition index**
799 **(Kn), glycogen content (µg mg^{-1} of wet mass), and total lipids (µg mg^{-1} of wet mass).** Mean ± SE. **Different letters indicate significant**
800 **differences among salinities**. FW: Freshwater preference; SW: Saltwater preference; BW: Brackish water preference; ns: no significant
801 difference.

	Mersey River - Date 1				**Mersey River - Date 2**			
	FW	**SW**	**BW**		**FW**	**SW**	**BW**	
	N=10	N=10	N=10		N=9	N=10	N=10	
Wet mass	0.17 ± 0.01	0.19 ± 0.01	0.17 ± 0.01	ns	0.22 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	Ns
Length	60.1 ± 1.31	59.8 ± 0.91	60.39 ± 1.49	ns	63.1 ± 0.76^{a}	61.6 ± 1.23^{ab}	59.3 ± 1.0^{b}	**p < 0.05**
Kn	1.02 ± 0.03^{ab}	1.13 ± 0.05^{a}	0.96 ± 0.05^{b}	**p < 0.05**	1.19 ± 0.04	1.13 ± 0.06	1.07 ± 0.05	ns
Glycogen	0.38 ± 0.07^{a}	0.18 ± 0.06^{ab}	0.12 ± 0.06^{b}	**p < 0.05**	0.31 ± 0.09	0.33 ± 0.09	0.22 ± 0.06	ns
Total lipids	6.75 ± 0.81	5.59 ± 0.79	11.41 ± 5.72	ns	7.20 ± 0.65	6.05 ± 0.72	5.81 ± 0.96	ns

	Grande-Rivière-Blanche - Date 1				**Grande-Rivière-Blanche - Date 2**			
	FW	**SW**	**BW**		**FW**	**SW**	**BW**	
	N=10	N=10	N=10		N=10	N=10	N=10	
Wet mass	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	ns	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	ns
Length	65.5 ± 0.80	65.9 ± 1.19	65.1 ± 1.09	ns	65.6 ± 1.30	65.8 ± 1.25	65.3 ± 1.31	ns
Kn	0.95 ± 0.04	0.90 ± 0.02	0.90 ± 0.03	ns	0.88 ± 0.03	0.87 ± 0.03	0.92 ± 0.03	ns
Glycogen	0.86 ± 0.08	0.89 ± 0.19	0.88 ± 0.08	ns	0.81 ± 0.17	0.67 ± 0.10	0.62 ± 0.09	ns
Total lipids	6.18 ± 0.38	5.85 ± 0.92	5.53 ± 0.50	ns	4.13 ± 0.40	5.56 ± 0.64	4.81 ± 0.58	ns

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